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Author(s)	Song, Isaiah Youhak
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氏名 Isaiah Youhak Song

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Physiological and genomic characteristics of *Eubacterium* sp. c-25 and their implications on the diversity of deoxycholic acid producers in the human gut

(*Eubacterium* sp. c-25 の生理学的およびゲノムの特徴とヒト腸内の
デオキシコール酸生産菌の多様性における意義)

Bile acids are secreted into the human gut to aid in the digestion of lipids and fat-soluble vitamins. A small amount of liver-derived primary bile acids are exposed to the biotransformative actions of the colonic microbiota and result in the formation of secondary bile acids (SBAs) especially through 7 α -dehydroxylation, which removes a hydroxy group at the C-7 position of primary bile acids. Deoxycholic acid (DCA) is produced from cholic acid (CA) as the most notable SBA and is known as a regulator of the gut microbiota and has been implicated in diseases such as colonic and hepatic cancer. While several 7 α -dehydroxylating bacteria have been identified, they are predominantly members or close relatives of the genus *Clostridium* and their taxonomic diversity is unknown.

Eubacterium sp. c-25 is a DCA-producing bacteria isolated and briefly studied in the 1980s that is poorly understood in the context of modern-day analytical techniques and knowledge of bile acid transformation. This study aims to characterize the physiology and genome of *Eubacterium* sp. c-25 against known DCA producers and extrapolate the data to explore the diversity of intestinal DCA producers and identify additional species with the potential to produce 7 α -dehydroxylated bile acids.

1. Comparative physiological and genomic characterization of *Eubacterium* sp. c-25 and *Clostridium scindens*

Eubacterium sp. c-25 exhibits a unique chain-like, filamentous morphology that is not found in other DCA producers. *In vitro* cultures of c-25 showed successful conversion of CA to DCA at a peak rate of ~50%, verifying the strain's 7 α -dehydroxylation ability. It was much lower than reference strains *C. scindens* G10 and *C. scindens* ATCC 35704^T, however, which were able to rapidly convert 80-90% of CA to DCA in ideal conditions. All three strains

exhibited a preference for pH 7 or 8 for maximum growth and DCA production.

The whole genome of c-25 was sequenced and revealed to consist of a 3,042,110 bp circular chromosome containing 2,893 coding sequences. It was found that the c-25 genome contained predicted orthologues of the bile acid-inducible (*bai*) genes necessary for 7 α -dehydroxylation. However, the unusual arrangement of the hypothesized *bai* genes into multiple clusters differed from the typical *bai* operon observed in *C. scindens* and other DCA producers. Several *bai* genes in c-25, specifically *baiB*, *baiCD*, and *baiH*, were selected for measurement of *in vitro* expression levels, and it was found that all three were upregulated in the presence of CA substrate, supporting their involvement in 7 α -dehydroxylation.

2. Exploration of *Eubacterium* sp. c-25 phylogeny and identification of additional 7 α -dehydroxylating bacteria

The amino acid sequence of BaiB from c-25 was used to search for strains that could share the same *bai* gene characteristics as c-25 and also produce DCA. Three additional strains were identified possessing genes sharing 74-77% sequence identity: *Sporofaciens musculi* (obese mouse cecal isolate), *Dorea* sp. AF36-15AT (human fecal isolate), and *Dorea* sp. AM58-8 (human fecal isolate). Comparative genomic analyses revealed that these three strains possessed *bai* genes that were arranged almost identically to c-25. A 16S rDNA phylogenetic tree analysis including these unconfirmed DCA producers suggested that 7 α -dehydroxylating bacteria are not restricted to certain evolutionary lineages, but are phylogenetically diverse.

In searching for marker genes that could be reliably indicative of 7 α -dehydroxylation ability, it was found that non-DCA producers consistently lacked orthologues of *baiE*, *baiI*, and *barB*. Using BaiE from c-25 and *C. scindens* G10 as queries, three additional species were detected as potential DCA producers in reference databases: *Proteocatella sphenisci* (penguin guano isolate), *Dorea* sp. D27 (human fecal isolate), and *Clostridium* sp. Marseille-P2538T (human fecal isolate).

In conclusion, this study revealed that *Eubacterium* sp. c-25 is a phylogenetically unique DCA producer possessing a novel arrangement of *bai* genes. While experimental evidence of DCA formation in the newly identified, unconfirmed DCA producers is necessary to verify *in silico* findings, the discovery of c-25 and its shared *bai* gene arrangement sets a precedent for the plausible existence of other genotypically non-traditional 7 α -dehydroxylating bacteria and further implies that DCA producer diversity in the human gut is greater than expected.